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YALE UNIVERSITY
OSBORN BOTANICAL LABORATORY
NEW HAVEN, CONNECTICUT

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Dear Mather-

The interest in our research which your letters indicate is most gratifying; under the circumstances, one could hardly consider you a tardy correspondent; I wish again to express again my appreciation for the stimulation which these epistolary conversations have provided.

Little progress has been made since my last letter, since neither of the two projects: crossover-suppressor search, nor 4-strand evidence has born any fruit. In re the latter, and in answer to your question, it would of course be necessary to recover more than a single product from the reduction of a zygote. Theoretically this might have been possible as follows: B_1- is a frequent segregant in the cross B-M-..X T-L-B_1-, to the point where most the of colonies which appear on B_1 supplemented plates will be B_1-. Since the manner in which the crosses are performed requires that the descendants of any cell be immobilized in the agar, in such B_1- colonies one might expect there to be a small number of cells complementary to the B_1- segregants which are the ones selected for in this medium. E.g., one might hope to find B-M-T-L-, or other combinations. They might be detected by scooping up the entire B_1- colony and plating it into medium lacking B_1 and containing the other factors. I have done this experiment a good many times, and have consistently recovered types other than the B_1- constituting the overwhelming major fraction of the colony. Unfortunately, these types have consisted exclusively of B-M- cells with the same LacV configuration as the parent, or of B_1+ types which undoubtedly arise by the (rare) reversion of the B_1 gene. The frequency of recombination (ca. 10^{-6}) is still so low as to exclude the chance of avoiding contamination of prototroph colonies with the parentals. Since these would be selected for equally as well or better than the hoped for complementary segregants, the failure to find the latter cannot be regarded as any indication of their true status. It may sometime be possible to find material in which the major segregant, and both parentals can be selected against, as would be demanded by this experiment.

As to crossover-suppression, I have been looking for types in which interchange between B_1 and B is inhibited. About 250 isolates of Nitrogen-mustard treated material have been tested, but none of these have shown any failure of interchange. As such, suppressors would not provide direct evidence of linear arrangement; however I hoped to have a sufficient collection of them involving divers portions of the region BM--TL as well to be able to map them linearly on this region. But nomluck at all yet.

On the question of the number of linkage groups, I have gone over the possibilities of 'spurious' linkage, and think the latter is excluded. The crucial data are perhaps the following. (all loci + unless indicated) (B-M- X T-L-B_1-) B-:B+ 11:79 T-:T+ 9:37 L-:L+ 5:51. These with the B_1 segregation are enough to exclude 'spurious' linkage. It is possible however, but unlikely that the reliability of these data is influenced by the chance of selection against B- types even on B containing media, etc. As it stands, however, all the data are explained on the basis of a single group, apropos which I might mention that the LacV segregation in the B- types supports the order B_1 --B---M--- rather than B_1 M----B

How far one should go in attempting a genetic analysis of this indirect sort is, it seems to me rather doubtful. I do not have stocks with which properly to perform the type of test for linear order which you suggested. Unreasonable assumptions of the absence of interference have had to be made because there is no direct test for it, and I wonder if the subject has not already been developed to the extent that it should be in the present state of our 'back ground' knowledge. For example, the question of 4-strand crossing over bears directly on the evaluation of linkage data. For example, in your analysis of the Lac, V segregation, you assigned the four classes the relative frequencies:

$p_1q_2q_3$ ..... and $p_1p_2p_3$.

However, 3- and 4- strand double exchanges also yield single exchange chromatids which should also be considered among the contributors to the first three classes. Finally, only a small fraction of triple exchanges will give rise to triple-echange chromatids for the fourth class. I have not attempted to arithmetize this analysis; perhaps you may have some suggestions.

In regard to the original analysis, it seems to me that the first three types should be in the ratio $p_1:p_2:p_3$ rather than $p_1q_2q_3:p_2q_1q_3:\ldots$ on the following basis. On that analysis, only single exchanges were considered for the first three types. In the absence of interference, the probabilities of interchanges in the class of single exchange types should be the same as for all types summed, i.e. proportional to the map distances. One can then use n_4 to estimate the absolute distances by $f(p_1+p_2+p_3)$ cubed is the probability of a triple crossover, of which a determinate fraction are of the type detected in n_4. What this will all look like on a four-strand basis is hard to foresee.

In the cross B-P- X T-L-B+ we may have the following situation.:

B_1	B	M	Lac	P	V	T-L
−	+	+	−	+	R	−
+	−	+	+	−	S	+

a b c d e

For a prototroph, exchange in region a and d or e is required → and no exchange in b or c
Lac-V^S and Lac-V^R. For Lac+ types, a double crossover in region b+c is needed.

For B_1^-, exchange in a is not necessary.

For B^-, one requires an exchange in b or c and in d or e

Then $B^-/B^+ = \dfrac{(P_b+P_c)(P_d+P_e)}{P_a\cdot(1-P_b-P_c)(P_d+P_e)}$.

Previous estimations of P_b+P_c (approx.) make $P_b+P_c \doteq 1-P_b+P_c$

I see here the point where my letter was confused, M did not enter into the cross, and on the basis of the map one should still expect that B- would be more frequent than B+. The map may be in error, or my statement that B- is not more frequent than B+ may be incorrect; I suspect the latter for this reason: the relative frequency of B- was in this case estimated from the relative number of colonies which appeared on B supplemented plates. It turns out that this is not a reliable procedure : for example, in the experiments in which B-:B+ was determined for the cross B-M- X T-L-B_1-, there were fewer colonies on B plates than on minimal , although there should have been an additional 12%. This probably stems from the fact that the background growth of the parental types is greater when B is supplied, and this may crowd out prototroph colonies. At all odds, this point will have to be looked into, and thanks for bringing it up.

Thank you also for transmitting the suggestion that we prepare some of our material for Heredity. I rather think that we will publish most of our experimental material in this country for reasons of convenience. However, I have been preparing an analysis of the literature on the 'genetic' peculiarities of bacteria, of which there are many which are perhaps not widely known (and authenticated, for that matter). I have not specifically intended to publish this material, but if the notion seems suitable, I should be happy to submit it.

My wife, Esther, extends her regards.

Yours sincerely,

Joshua Lederberg.

Giving a critical analysis